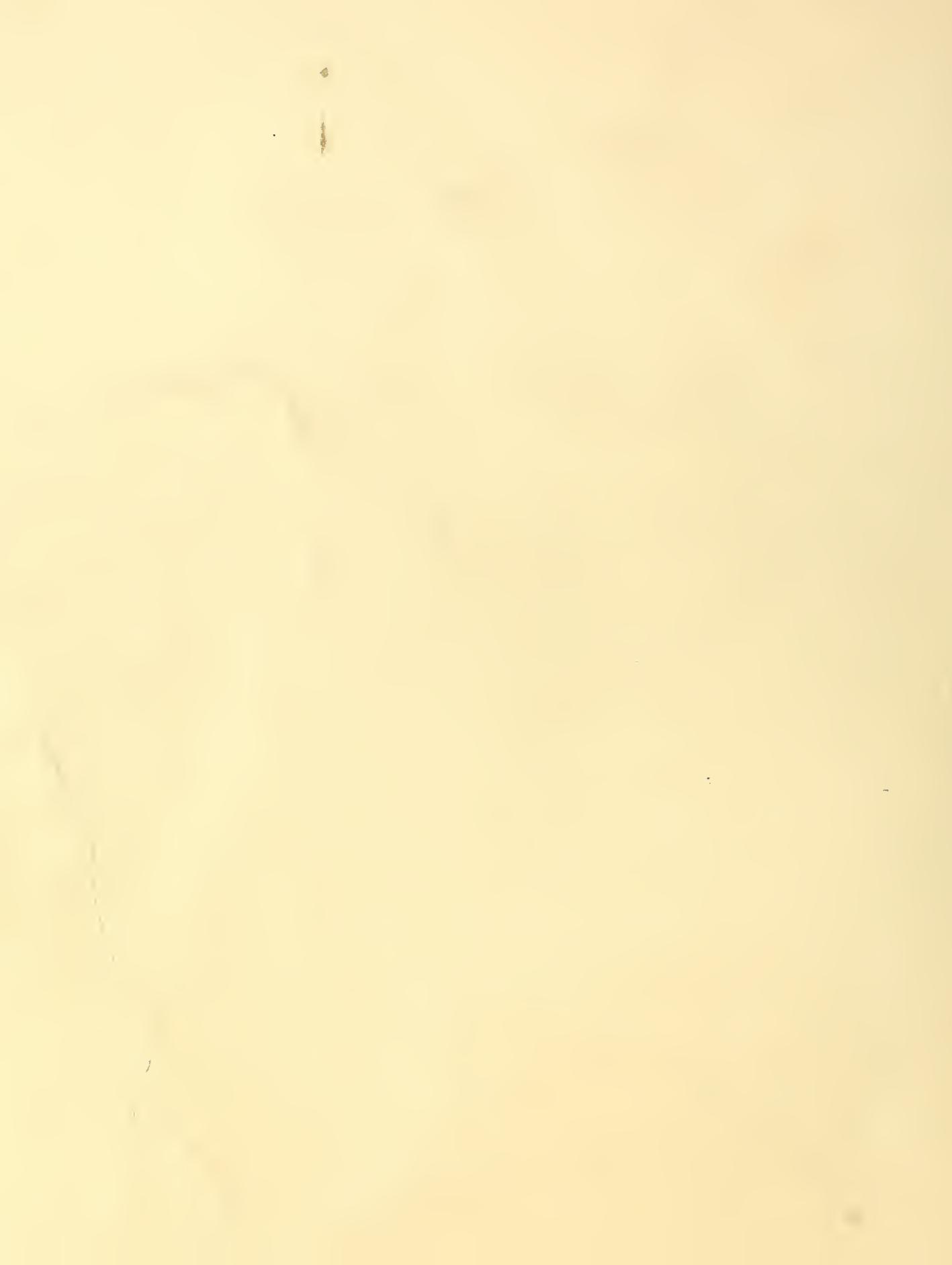


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RAPID

PEROXIDASE TEST

for better control of blanching



**Agricultural
Research
Service**

U. S. DEPARTMENT OF AGRICULTURE

THE PEROXIDASE TEST PAPER described here promises to be very useful in processing plants.

With a little experience a quality-control operator can determine whether or not blanching is uniform--simply by pressing selected peas, corn or other vegetables against the paper and waiting a minute or more for the color reaction. By pressing cut surfaces of large pieces against the paper he can discover depth of inactivation of peroxidase. With a stopwatch he can make reliable quantitative measurements of adequacy of heat treatment.

Those who use this test paper may become interested in additional technical information, which can be obtained from the address below.

This information was prepared in the
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RAPID PEROXIDASE TEST FOR BETTER CONTROL OF BLANCHING

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This report describes an easily made test paper that reveals extent of inactivation of the enzyme peroxidase within a few minutes. Quality-control workers can prepare a supply of test papers without excessive labor and without elaborate equipment. Comparisons with other methods have shown that this test paper is highly reliable. Ease of preparation and speed of reaction make it highly advantageous for commercial use.

The need for inactivation of enzymes can be explained briefly. After harvest and during processing, undesirable changes may occur in plant material. Desirable substances, for example vitamins, may be destroyed or substances may be produced that cause objectionable changes in color or flavor. Both types of change are catalyzed by naturally occurring enzymes. To prevent or reduce these changes, it is common practice to subject the fresh tissue to either steam or hot-water blanching. By this means the enzymes are inactivated quickly, thus preventing their catalytic action. Excessive blanching may be objectionable, however, from the standpoint of product quality.

For control of the blanching process tests are necessary to reveal the progress or extent of enzyme inactivation. One of the most heat-stable enzymes, peroxidase, is also one of the easiest to detect or measure. These factors, coupled with its almost universal presence in plant material, make peroxidase a very convenient enzyme for use in evaluating the extent of blanching in many products.

Earlier work in this Laboratory on tests for adequacy of blanching can be reviewed briefly as follows. In 1944, Masure and Campbell (Fruit Prod. Jour. 23:369) published reliable chemical methods--both quantitative and semiquantitative. In 1945 Morris and Lineweaver published a test for dehydrated white potatoes (Food Packer 26(1):40) that was adopted as the official test by the Office of the Quartermaster General. This method is used by some potato processors. In 1953 Masure, Dietrich, Lindquist, and Blackwood published a test especially adapted to Brussels sprouts (Food Technol. 7:363).

More recently (1955) Dietrich, Lindquist, Bohart, Morris, and Nutting (Food Research 20:480) published results of extensive studies of inactivation of catalase and peroxidase in frozen peas, which showed that peas heat-treated just adequately for a negative peroxidase test maintained better flavor and color and retained more ascorbic acid than samples blanched for shorter or longer times.

The Masure-Campbell tests are reliable but require much more time than use of the ready-made test paper. Steps to follow in the preparation and use of test papers are presented below. Various products are mentioned by commercial name for purposes of illustration, but such use of names is not intended to imply recommendation of these products over others of similar nature.

MATERIALS NEEDED

Filter paper. Whatman's Nos. 1 and 50 and Carl Schleicher and Schnell Company's Nos. 576, 595, and 597 have all been used successfully. Heavy paper like Whatman No. 3 is not recommended.

Ortho-tolidine, reagent quality.

Urea peroxide (available from Becco Chemical Division, Food Machinery and Chemical Corporation, Buffalo 7, New York).

95 percent ethyl alcohol.

C-clamp, heavy duty, 6- to 8-inch size.

Two stainless-steel plates. Convenient size is $4 \times 4 \times 1/4$ inches.

Tool for making 3/8-inch hole in paper.

Stop watch.

Calcium chloride, anhydrous (desiccant).

Beakers.

Wide-mouth Mason jars for storage of papers.

PREPARATION OF REAGENTS

Prepare fresh reagents before use.

Ortho-tolidine. Prepare a one percent solution by dissolving 0.5 gram of ortho-tolidine in 50 ml. of alcohol.

Urea peroxide. Prepare a one percent solution by dissolving 0.5 gram of urea peroxide in 50 ml. of alcohol.

Combined reagent. Immediately before use mix equal volumes of one percent alcoholic solutions of ortho-tolidine and urea peroxide.

PREPARATION OF PAPER

Punch 3/8-inch holes near the edge of a stack of filter papers. Many tools can be used. A Whitney-Jensen bench punch press has been used, in which 40 papers can be punched at one time.

Arrange 50 to 100 filter papers in a neat stack and place them in a beaker just large enough in diameter to accommodate the papers without folding. Cover the papers with the combined reagent and allow to stand for 30 to 60 seconds, or until all papers are saturated. Decant the surplus reagent solution, remove the stack of papers from the beaker with minimum disturbance, and place between the two stainless-steel plates.

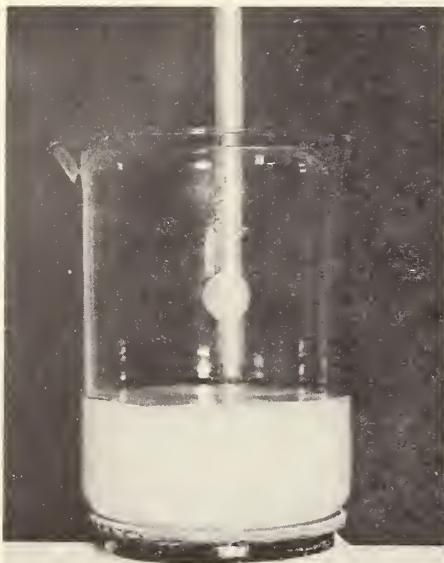
Apply very firm pressure by tightening the C-clamp securely against the plates. Holding the C-clamp in a vise during this operation makes the procedure much simpler than holding the clamp in one's hands. In pressing the papers one is more likely to err by not using enough pressure than by using too much. If too little pressure is used, too much reagent will remain on the filter papers, causing them to turn slightly blue on drying. Hold the papers under pressure for about 30 seconds to allow the maximum amount of solution to be removed.

Suspend the treated papers on a string and space them 1 to 2 inches apart. Allow 30 minutes for the residual alcohol in the paper to evaporate. The moisture content of the finished papers is a factor of considerable importance in relation to stability of the paper. For this reason the papers should be prepared under as low a humidity as is practical. The papers should also be protected from direct sunshine or other bright lights.

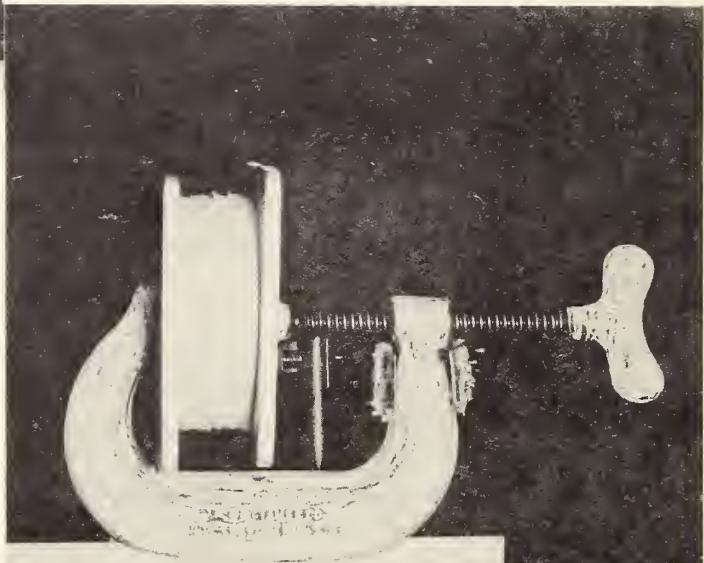
Collect the finished test papers and store them in a desiccated atmosphere under refrigeration. Routine practice has been to place 50 to 100 grams of anhydrous calcium chloride in a wide-mouthed fruit jar, cover the calcium chloride with a piece of cotton, put 20 to 30 of the finished test papers on top of the cotton, close the jar, and store it in a refrigerator.

STABILITY OF TEST PAPERS

The test papers are perishable. Their useful life depends on the storage conditions. The two most important factors affecting their stability are humidity and temperature. Test papers that are properly desiccated and given refrigerated storage remain in good condition for several months. Papers stored in freezers should be useful for several years. Under such conditions one should be careful that the papers are not permitted to increase in moisture content, due to condensation, when they are removed for use. It is best to allow the jar to reach room temperature before it is opened.

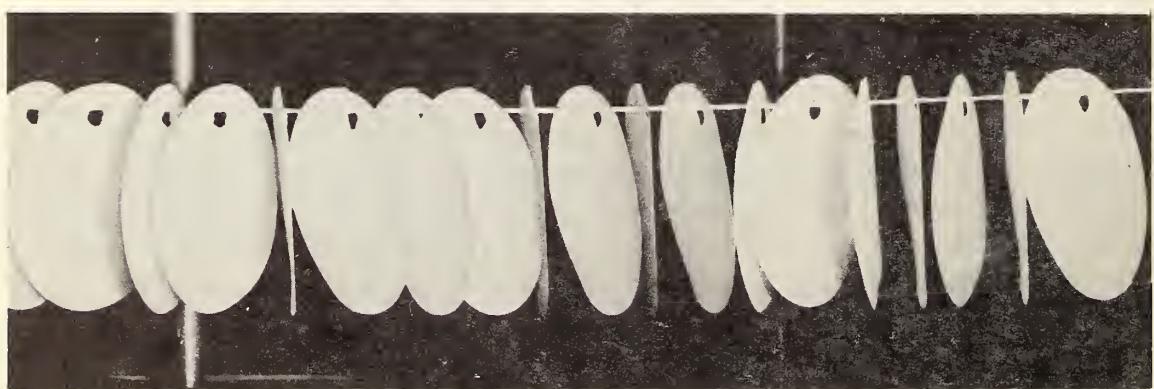


Adding
reagents
to paper



Removing
excess
reagent
solution

Residual solvent evaporation





Peroxidase test
using vegetable extract



Peroxidase test
using pieces of vegetable

Desiccated papers kept in the dark at room temperature can be used after several days of storage; however, room-temperature storage should be avoided for papers that are to be kept for more than a week.

USE OF THE TEST PAPER

The test paper is used in two ways: (a) a small drop of vegetable extract is placed on the test paper, or (b) the test paper is pressed firmly against a freshly cut surface of a solid piece of plant tissue. These methods supply information that helps the quality-control man in his efforts to evaluate the effectiveness of the blanching step.

Extract Method

Tests made with an extract reflect the average peroxidase activity of all the sample used. The time required for a positive reaction, as revealed by a definite blue color on the test paper, is inversely proportional to the activity present. The time for a blue color to appear is measured by starting a stopwatch when the drop of extract is added to the paper and stopping it when a definite blue color is observed. The extent of color formation selected as representing a positive reaction is necessarily somewhat arbitrary, but the same intensity of color can be used in all tests.

With some extracts the blue color appears first along the edge of the moistened area. In these instances the intensity of color along the edge determines when the test is considered positive. The reason for this behavior in color pattern is not understood, but it is encountered most frequently with unbuffered extracts.

The "end-point" or time required for adequately blanched vegetables will vary within narrow limits, because products, processing conditions, and papers will vary slightly. Under laboratory conditions, however, the results obtained on peas, cauliflower, broccoli, and asparagus indicate that all samples that required one minute or more to give a positive reaction were adequately blanched as judged by results of the qualitative Masure and Campbell test on the same samples. Some processors refer to this qualitative test as the "U. S. D. A. Method". This value (one minute) should be used only as a general guide to help orient the operator in the use of the paper under his experimental or plant conditions.

For most control work, extracts can be prepared by blending the samples with distilled water. Greater precision is obtained, however, if the extracts are prepared with pH 5 buffer solutions.

Water extract. Blend a representative sample, 50 to 100 grams, with 3 times its weight of water in an electrical blender for 2 minutes.

Buffered extract. Blend a representative sample, 50 to 100 grams, with 3 times its weight of 0.01M acetate buffer at pH 5.0 for 2 minutes. The buffer is prepared by mixing 0.01M solutions of acetic acid and sodium acetate in the ratio of 1.0 to 1.5.

The extracts can be filtered through a gauze-back cotton milk filter, but filtration is optional. It is possible to make the tests on unfiltered extracts.

Solid Pieces

Results obtained with a freshly cut surface of a solid piece show the relative peroxidase activities of portions of the individual piece. For example, if one makes tests on individual pieces of cauliflower or broccoli it is possible to show that the centers of the large pieces sometimes give strong positive tests, whereas the outer portions are free of peroxidase activity. With vegetables like corn and peas, tests on individual pieces reveal any variations that exist in the product.

Tests on solid pieces eliminate the preparation of extracts, but the results must be evaluated in terms of the specimen tested. For example, a positive reaction observed on an extra large piece of blanched vegetable does not necessarily indicate that smaller pieces would give positive reactions. On the other hand, variations in the activities of similar-sized pieces could result from lack of uniform blanching conditions. In that case results on individual pieces could help a processor recognize a situation that would not be so readily apparent by tests made on extracts.

